Nonalcoholic Fatty Liver Disease Is Prevalent in Women With Prior Gestational Diabetes Mellitus and Independently Associated With Insulin Resistance and Waist Circumference

Signe Foghsgaard,[1–3] Camilla Andreasen,[1] Louise Vedtofte,[1] Emilie S. Andersen,[1] Emilie Bahne,[1] Charlotte Strandberg,[4] Thora Buhl,[5] Jens J. Holst,[2] Jens A. Svare,[6] Tine D. Clausen,[7] Elisabeth R. Mathiesen,[8] Peter Damm,[9] Lise L. Gluud,[1] Filip K. Knop,[1,2] and Tina Vilsbøll[1]

Diabetes Care 2017;40:109–116 | DOI: 10.2337/dc16-1017

OBJECTIVE

Type 2 diabetes increases the risk of nonalcoholic fatty liver disease (NAFLD), which is a potentially reversible condition but is also associated with progressive fibrosis and cirrhosis. Women with prior gestational diabetes mellitus (pGDM) have a higher risk for NAFLD.

RESEARCH DESIGN AND METHODS

One hundred women without diabetes who had pGDM (median [interquartile range]: age 38.6 [6.4] years; BMI 31.0 [6.2] kg/m^2) and 11 healthy control subjects without NAFLD (age 37.9 [7.8] years; BMI 28.1 [0.8] kg/m^2) underwent a 75-g oral glucose tolerance test (OGTT), DXA whole-body scan, and ultrasonic evaluation of hepatic steatosis.

RESULTS

Twenty-four (24%) women with pGDM had NAFLD on the basis of the ultrasound scan. None had cirrhosis. Women with NAFLD had a higher BMI (P = 0.0002) and waist circumference (P = 0.0003), increased insulin resistance (P = 0.0004), and delayed suppression of glucagon after the OGTT (P < 0.0001), but NAFLD was not associated with the degree of glucose intolerance (P = 0.2196). Visceral fat mass differed among the three groups, with the NAFLD group having the highest amount of fat and the control subjects the lowest (P = 0.0003). By logistic regression analysis, insulin resistance (P = 0.0057) and waist circumference (P = 0.0109) were independently associated with NAFLD.

CONCLUSIONS

NAFLD was prevalent in this cohort of relatively young and nonseverely obese women with pGDM who are considered healthy apart from their increased risk for diabetes. Insulin resistance and a larger waist circumference were independently associated with the presence of NAFLD, whereas glucose intolerance was not.

[1]Center for Diabetes Research, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark
[2]Novo Nordisk Foundation Center for Basic Metabolic Research and Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Hellerup, Denmark
[3]Danish Diabetes Academy, Odense University Hospital, Odense, Denmark
[4]Department of Radiology, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark
[5]Department of Nuclear Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark
[6]Department of Obstetrics and Gynecology, Herlev Hospital, University of Copenhagen, Herlev, Denmark
[7]Department of Gynecology and Obstetrics, Nordsjællands Hospital, University of Copenhagen, Hillerød, Denmark
[8]Center for Pregnant Women with Diabetes, Department of Endocrinology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[9]Center for Pregnant Women with Diabetes, Department of Obstetrics, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Corresponding author: Tina Vilsbøll, t.vilsboll@dadlnet.dk.

Received 10 May 2016 and accepted 10 October 2016.

Clinical trial reg. no. 2012-001371-37, clinical trialsregister.eu.

S.F. and C.A. are co-first authors.

Nonalcoholic fatty liver disease (NAFLD) is hepatic accumulation of triglycerides in the absence of excessive alcohol consumption (1). NAFLD is the most common liver abnormality in the Western world, with a prevalence of 20–33% in the general European population of adults and 43–70% in patients with type 2 diabetes (T2D) (2). NAFLD spans from simple steatosis to the more aggressive form nonalcoholic steatohepatitis (NASH) that may progress to fibrosis, cirrhosis, and end-stage liver failure (3). Visceral, as opposed to subcutaneous, adipose tissue is especially unhealthy because of its metabolically active nature and because it releases free fatty acids directly into the portal venous system (4–6). The majority of patients with mild to moderate NAFLD without NASH are asymptomatic, and in up to 70% of these patients, no abnormalities in plasma liver enzyme levels are observed (3,4,7). NAFLD is associated with hyperglucagonemia, visceral adiposity, obesity, insulin resistance, and T2D, the latter three of which are features of the metabolic syndrome (3,8,9). Of note, both NAFLD and T2D are associated with a markedly increased risk of cardiovascular disease (3,5,7). Liver biopsy is the gold standard for diagnosing NAFLD and necessary for the diagnosis of NASH, but imaging methods are increasingly being accepted as noninvasive alternatives because the invasive nature of biopsy has several important disadvantages (10).

Gestational diabetes mellitus (GDM) is glucose intolerance first detected during pregnancy and affects 2–6% of pregnant European women (11). In the majority of women, normal glucose tolerance (NGT) is reestablished after delivery (12,13). Nevertheless, women with prior GDM (pGDM) may progress to overt T2D, with a long-term risk of up to 70% (14), and even if NGT is maintained, these women are more prone to the metabolic syndrome than those who had NGT during pregnancy (15). Additionally, women with pGDM have a twofold risk of NAFLD compared with women without a history of GDM, even when adjusted for BMI (16). Because of their increased risk, women without diabetes but with pGDM represent a valuable target group for investigating the early metabolic changes that precede T2D. In this study, we investigated the presence of NAFLD and its association with glucose intolerance, insulin resistance, and fat distribution in a cohort of overweight or obese women without diabetes but with pGDM (16).

RESEARCH DESIGN AND METHODS

Study Design

The study included baseline data from women who were recruited for an investigator-initiated, randomized, placebo-controlled, double-blind intervention trial carried out in women without diabetes but with pGDM before the intervention was initiated (17). The protocol was approved by the Danish Data Protection Agency (01714 GEH-2012-024) on 4 June 2012, the Danish Medicines Agency (EudraCT no. 2012-001371-37) on 10 July 2012, and the Scientific-Ethical Committee of the Capital Region of Denmark (H-2-2012-073) on 13 July 2012. The study was carried out under the surveillance of the Good Clinical Practice unit (Copenhagen, Denmark) and conducted in accordance with the Declaration of Helsinki.

Outcomes

The primary outcome was the presence of ultrasound-detectable NAFLD in a cohort of women with pGDM.

Participants and Recruitment

One hundred five women without diabetes but with pGDM were recruited to the main study (17), and of these, 100 underwent B-mode ultrasonographic evaluation of the liver for the presence or absence of NAFLD. The trial allowed inclusion of all ethnicities, but only 4 of the 100 women were non-Caucasian (one African and three Asian). We did not include women with known liver disease (based on patient history and biochemical and ultrasonic assessment), increased liver enzymes, or ongoing alcohol abuse. Four women presented with levels of liver enzymes above normal limits (but below three times the normal limit). These women were examined with concern for hepatitis B and C with negative results. Eleven healthy women without ultrasound-detectable NAFLD, pGDM, or glucose intolerance were included as the control group (17). The evaluated groups were *1*) women without pGDM and without NAFLD (control), *2*) women with pGDM without NAFLD (nonNAFLD pGDM), and *3*) women with pGDM and NAFLD (NAFLD pGDM).

After signed consent, a screening visit was completed wherein fasting blood samples for creatinine, sodium, potassium, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, albumin, hemoglobin, fasting plasma glucose, and glycated hemoglobin A_{1c} (HbA_{1c}) levels were collected to verify that the participant fulfilled all the inclusion criteria and none of the exclusion criteria (17). Medical history was recorded, and a full physical examination was performed.

Procedure

On the experimental days, participants came in the morning after a 10-h fast and underwent a number of examinations (17).

Ultrasonography

Real-time B-mode ultrasonography of the liver was performed with a high-end ultrasound scanner (Logiq E9; General Electric, Milwaukee, WI), using a convex probe (2.5–6 MHz). All examinations were performed by the same specialized radiologist (C.S.).

Transient Elastography

To rule out hepatic fibrosis, vibration-controlled transient elastography (FibroScan; Echosens, Paris, France) was performed by one of two trained investigators (S.F., L.V.). All transient elastography results with an interquartile range (IQR) $<$30% of the median value and a success rate of at least 60% were analyzed. A median of hepatic elasticity $>$8 kPa implied hepatic fibrosis (18).

DXA

Body composition and fat distribution measures were acquired using Lunar iDXA (GE Healthcare, Chicago, IL) and analyzed with the accompanying software enCORE version 13.6, where visceral fat mass was computed by subtracting subcutaneous fat mass from total abdominal fat mass in the predefined android region (19).

Oral Glucose Tolerance Test

A 4-h 75-g oral glucose tolerance test (OGTT) was performed to define glucose tolerance status and to rule out diabetes. Women were categorized as having NGT or prediabetes according 2006 World Health Organization criteria (20). Whole-body insulin resistance was calculated according to the Matsuda index (21), and hepatic insulin resistance was evaluated with the computerized HOMA2 for insulin resistance ($HOMA2_{IR}$) (22). The insulinogenic index was calculated as the

ratio between the total area under the curve (tAUC) of serum insulin and plasma glucose during the OGTT (23). The disposition index (insulinogenic index / $HOMA2_{IR}$) was used as an adjusted measure of β-cell function (23). Presence of the metabolic syndrome was assessed by joint scientific statement criteria (24).

Biochemical Markers

Liver function was evaluated by biochemical markers (ALT, AST, and γ-glutamyl transferase [GGT]). Women with ALT and AST levels three times above normal limits were excluded according to protocol (17). The probability score of steatosis was calculated according to the recently validated fatty liver index (FLI), categorizing the women into three groups: G1 with FLI ≤30 (very-low risk of steatosis); G2 with FLI >30 and <60 (intermediate risk of steatosis); and G3 with FLI ≥60 (high risk of steatosis) (25).

Alcohol Consumption

Habits were evaluated through a validated questionnaire, the Alcohol Use Disorder Identification Test (AUDIT), to rule out excessive alcohol consumption (score <8) (26).

Analysis

Plasma glucose (27), glucagon (28,29), and insulin and C-peptide (30) levels were analyzed as previously described.

Statistics

Data are tabulated as median and IQR or number and percent. Fasting values of glucose, insulin, C-peptide, and glucagon were calculated as the mean of −15, −10, and 0 min. The AUC was calculated by the trapezoidal rule and expressed as either the tAUC or the incremental AUC (iAUC) for all 240 min, unless otherwise stated. The statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA) and RStudio version 0.98.1083 (RStudio, Boston, MA) software. Bartlett test was used to assess for normal distribution. Comparisons among the three groups were performed using Kruskall-Wallis test with Dunn correction for multiple comparisons for continuous variables. Differences of categorical variables were analyzed using χ^2 test. Logistic regression analysis of the significant variables in the univariate regression analysis were used to identify clinically relevant determinants associated with the presence of NAFLD. Differences resulting in $P < 0.05$ were considered significant.

RESULTS

Baseline Characteristics

Anthropometric and metabolic characteristics of the control (n = 11), nonNAFLD pGDM (n = 76), and NAFLD pGDM (n = 24) groups are listed in Table 1. The three groups were similar with regard to age, waist-to-hip ratio, heart rate, HbA_{1c}, android-to-gynoid fat ratio, AUDIT scores, and AST-to-ALT ratio as well as with regard to use of hormonal contraception, polycystic ovarian syndrome, number of pregnancies, and time since last pregnancy. BMI was highest in the NAFLD pGDM group. Systolic and diastolic blood pressure was lowest in the control group. Total cholesterol and LDL were similar among groups, but HDL was lower in the NAFLD pGDM group, and VLDL and triglycerides were lower in the control group. DXA scan revealed different amounts of visceral fat in the three groups, with the control group being the leanest and having the lowest total fat mass. GGT was similar and within the normal range in all three groups, whereas ALT was higher in the NAFLD pGDM group. Four women in this group had values that exceeded the upper limit of normal (35 units/L) but less than three times this. AST was higher in the NAFLD pGDM group than in the nonNAFLD pGDM group, but all three groups were well within the normal range. FLI was higher in the NAFLD pGDM group, with 88% of women categorized as G3 (high risk of steatosis) (Table 1). No differences were observed with respect to ethnicity.

Glucose Tolerance and Indices of Insulin Resistance

Data from the OGTT are listed in Table 2; the dynamic responses of glucose, glucagon, insulin, and C-peptide are illustrated in Fig. 1*A*–*D*. Fasting plasma glucose, the 2-h value, and the peak value and tAUC for plasma glucose excursions were lower in the control group. No differences between the pGDM groups were observed, and no differences with respect to prevalence of prediabetes were found. Although there was a numerical tendency, fasting plasma glucagon did not differ significantly among groups, but during the first 45 min after glucose ingestion, both tAUC and iAUC were higher in the NAFLD pGDM group. Fasting serum insulin was higher in NAFLD pGDM, but no difference between nonNAFLD pGDM and control was found. The tAUC for serum insulin differed among the three groups, with the largest being in the NAFLD pGDM group and the smallest being in the control group. C-peptide followed the same pattern, with higher fasting serum C-peptide in the NAFLD pGDM group and increasing tAUC for serum C-peptide across the three groups, the largest being in the NAFLD pGDM group. The insulinogenic index was higher in the NAFLD pGDM group, whereas the disposition index was similar in all three groups. The Matsuda index was lowest in the NAFLD pGDM group and highest in the control group. Similarly, $HOMA2_{IR}$ was highest in the NAFLD pGDM group.

Logistic Regression Analysis

Univariate logistic regression analysis of women with pGDM showed several determinants to be significantly associated with the presence of NAFLD (Table 3). The following variables did not reach significance: glucose tolerance status, age, waist-to-hip ratio, HbA_{1c}, 2-h plasma glucose during OGTT, fasting plasma glucagon, family disposition to diabetes, use of hormonal contraceptive, total cholesterol, LDL cholesterol, AUDIT score, and presence of the metabolic syndrome. All significant univariable associations contributed in the multivariable analysis with backward elimination, showing that waist circumference (P = 0.011) and Matsuda index (P = 0.006) were independent determinants associated with NAFLD (Table 3).

CONCLUSIONS

We show that NAFLD is present in women without diabetes but with pGDM. Our thorough examination of glucose dynamics and metabolism showed no association with the degree of glucose intolerance, whereas increasing insulin resistance and larger waist circumference were independently associated with the presence of NAFLD. When comparing the NAFLD pGDM group with the control group, we found that body composition was significantly more android, with a larger waist circumference, more visceral fat mass, and greater total fat mass. We found no differences in fasting levels of plasma glucagon, but the suppression of plasma glucagon after oral glucose ingestion was significantly delayed, and the initial suppression was reduced in women with NAFLD and pGDM.

Table 1—Anthropometric, metabolic, and ultrasonic characteristics and indices of insulin resistance and β-cell function

	Group			*P* value		
	Control (A)	nonNAFLD pGDM (B)	NAFLD pGDM (C)	A – B	A – C	B – C
Number of participants	11	76	24			
Age (years)	37.9 (7.8)	39.0 (5.6)	36.9 (5.6)	>0.0999	>0.0999	0.2945
BMI (kg/m^2)	28.1 (0.8)	29.9 (4.7)	34.6 (4.7)	0.3629	0.0003	0.0002
Waist circumference (cm)	98.0 (14.0)	101 (16)	109 (17)	0.2009	0.0001	0.0003
Waist-to-hip ratio	0.9 (0.0)	0.9 (0.0)	0.9 (0.1)	>0.9999	>0.9999	>0.9999
Systolic blood pressure (mmHg)	116 (14)	127 (15)	128 (13)	0.0166	0.0500	>0.9999
Diastolic blood pressure (mmHg)	76 (11)	80 (14)	82 (7)	0.0439	0.0268	>0.9999
Heart rate (beats/min)	72 (12)	68 (14)	74 (11)	>0.9999	>0.9999	0.4093
HbA_{1c} (mmol/mol)	31.0 (4.5)	33.0 (5.0)	34.0 (7.5)	0.3349	0.0732	0.5721
Metabolic syndrome	1 (9)	35 (46)	15 (63)	0.0131	0.0131	0.0131
Use of hormonal contraception	5 (45)	47 (62)	15 (63)	0.8889	0.8889	>0.9999
Pregnancies	2.0 (1.5)	2.0 (1.5)	2.0 (0.0)	>0.9999	>0.9999	>0.9999
Time since pregnancy (years)	4.5 (4.5)	4.8 (4.2)	4.5 (2.6)	>0.9999	>0.9999	>0.9999
Polycystic ovarian syndrome	*	4 (5.3)	4 (17)	*	*	0.0726
Total cholesterol (mmol/L)	4.4 (0.9)	4.7 (1.2)	5.0 (0.9)	0.7034	0.1106	0.3280
HDL cholesterol (mmol/L)	1.4 (0.6)	1.2 (0.3)	1.1 (0.4)	0.0813	0.0022	0.0081
LDL cholesterol (mmol/L)	2.8 (1.0)	3.2 (1.2)	3.3 (0.7)	0.7108	0.1624	0.5165
VLDL cholesterol (mmol/L)	0.3 (0.1)	0.5 (0.2)	0.6 (0.5)	0.0266	0.0030	0.3600
Triglycerides (mmol/L)	0.7 (0.1)	1.0 (0.6)	1.3 (1.0)	0.0144	0.0006	0.1640
Visceral fat (g)	375 (113)	908 (771)	1,469 (896)	0.0094	<0.0001	0.0003
Android-to-gynoid fat ratio	1.0 (0.1)	1.1 (0.2)	1.1 (0.2)	>0.9999	>0.9999	>0.9999
Fat mass (%)	39.5 (1.0)	43.7 (7.5)	46.4 (6.9)	0.0253	0.0012	0.1846
AUDIT score	3.0 (3.0)	3.0 (2.0)	2.0 (1.0)	>0.9999	>0.9999	>0.9999
GGT (units/L)	16.5 (5.8)	18.0 (10.0)	20.0 (8.3)	>0.9999	>0.9999	0.8332
ALT (units/L)	21.0 (7.0)	22.0 (9.5)	27.5 (6.5)	>0.9999	0.0591	0.0037
AST (units/L)	27.0 (9.5)	25.0 (6.0)	27.0 (8.5)	>0.9999	>0.9999	0.0232
AST-to-ALT ratio	1.2 (0.8)	1.1 (0.3)	1.0 (0.5)	>0.9999	0.1762	0.2315
FLI	36.7 (18.8)	50.4 (41.9)	85.2 (20.2)	0.1929	<0.0001	0.0001
E-median (kPa)	4.7 (1.7)	3.9 (1.1)	5.5 (2.1)	0.3205	0.7638	0.0002
Insulinogenic index	0.3 (0.1)	0.3 (0.2)	0.7 (0.5)	0.3521	0.0010	0.0019
$HOMA2_{IR}$	1.2 (0.6)	1.5 (0.8)	2.4 (1.2)	0.4226	<0.0001	<0.0001
Disposition index	0.2 (0.1)	0.2 (0.2)	0.3 (0.4)	>0.9999	0.5779	0.0981
Matsuda index	5.2 (1.7)	2.8 (1.9)	1.5 (1.2)	0.0155	<0.0001	0.0004

Data are median (IQR) for continuous variables and *n* (%) for categorical variables. Continuous variables were analyzed using the Kruskall-Wallis test with Dunn correction for multiple comparisons. Differences of categorical variables were analyzed by χ^2 test with Bonferroni test for multiple comparisons. $P < 0.05$ was considered significant. Disposition index, insulinogenic index/$HOMA2_{IR}$; E-median, median of hepatic elasticity as measured by vibrant-controlled transient elastography; Matsuda index, $10{,}000/\sqrt{}$(fasting glucose × fasting insulin) × (mean glucose × mean insulin). *Polycystic ovarian syndrome was an exclusion criterion for control subjects.

A limitation to this study is the use of ultrasound to determine the presence or absence of NAFLD. Ultrasonography does not detect mild steatosis (5–33% fat infiltration) and is operator dependent (5). We accommodated the latter by letting one specialized radiologist (C.S.) perform and describe all examinations. Liver biopsy remains the gold standard for grading and staging NAFLD (10), but for ethical and practical reasons, liver biopsy was not performed. Thus, we may not have detected the presence of mild cases of NAFLD in this cohort, but whether detection of these stages of NAFLD has a clinical significance is debatable. Two studies have shown that simple steatosis does not necessarily progress to NASH and more severe liver damage (2,6). The median FLI in the nonNAFLD pGDM group indicates that a large percentage of these women have mild steatosis. Indeed, only 13 of 76 women had an FLI <30 (25). To our knowledge, only two previous studies have investigated the prevalence of NAFLD in women with pGDM (16,31). The first study was cross-sectional and showed a higher prevalence of ultrasound-detectable NAFLD among European women with pGDM (38%) compared with the current findings (24%) (16). The difference in reported prevalence does not seem to be explained by differences in age, BMI, body composition, fat percent, years since index pregnancy, ethnicity, means of detecting NAFLD, or size of study cohort and might instead be attributed to chance. An equal and important limitation of this study is the relatively small sample size, but despite this, we were able to demonstrate clinically relevant differences among the groups. The second study is a recent subgroup analysis

Table 2—Glucose data

	Group			*P* value		
	Control (A)	nonNAFLD pGDM (B)	NAFLD pGDM (C)	A – B	A – C	B – C
Glucose baseline (mmol/L)	4.9 (0.5)	5.4 (0.5)	5.4 (0.8)	0.0095	0.0108	>0.9999
Glucose peak (mmol/L)	8.2 (1.6)	10.0 (2.1)	10.7 (1.6)	0.0003	0.0002	>0.9999
2-h plasma glucose (mmol/L)	7.0 (0.4)	8.0 (2.1)	8.1 (2.1)	0.0031	0.0003	0.4103
Glucose tAUC (mmol/L × min)	1,433 (178)	1,668 (245)	1,764 (356)	0.0004	<0.0001	0.2196
Glucose iAUC (mmol/L × min)	203 (66)	389 (226)	466 (215)	0.0056	0.0004	0.3000
Prediabetes	*	46 (61)	17 (71)	*	*	0.5031
Insulin baseline (pmol/L)	54.2 (29.5)	78.6 (42.7)	127 (59)	0.3029	<0.0001	<0.0001
Insulin tAUC (nmol/L × min)	49.1 (21.3)	73.7 (57.3)	157 (101)	0.0308	<0.0001	0.0009
Insulin iAUC (mmol/L × min)	34.4 (14.9)	57.0 (48.4)	122 (93)	0.0111	<0.0001	0.0043
C-peptide baseline (pmol/L)	372 (143)	503 (237)	664 (199)	0.1586	0.0001	0.0005
C-peptide tAUC (nmol/L × min)	241 (138)	352 (122)	468 (168)	<0.0001	<0.0001	0.0164
C-peptide iAUC (nmol/L × min)	163 (79)	223 (92)	265 (136)	<0.0001	<0.0001	0.1757
Glucagon baseline (pmol/L)	5.0 (3.8)	6.0 (3.1)	6.8 (2.2)	>0.9999	0.3305	0.3027
Glucagon tAUC (0–45) (pmol/L × min)	178 (132)	258 (112)	1,038 (386)	0.5892	<0.0001	<0.0001
Glucagon iAUC (0–45) (pmol/L × min)	−66.3 (49.0)	−10.0 (76.0)	730 (389)	0.0875	<0.0001	<0.0001

Data are median (IQR) for continuous variables and *n* (%) for categorical variables. Continuous variables were analyzed using the Kruskall-Wallis test with Dunn correction for multiple comparisons. Differences of categorical variables were analyzed by χ^2 test with Bonferroni test for multiple comparisons. $P < 0.05$ was considered significant. *Prediabetes was an exclusion criterion for control subjects.

of the longitudinal Coronary Artery Risk Development in Young Adults cohort (31). The subgroup comprised black and white Americans with (*n* = 124) and without (*n* = 991) self-reported GDM. The women underwent computed tomography quantification 25 years after entry into the study. The study found a prevalence of 14% in the pGDM group and 5.8% in the non-GDM group. The strong association between NAFLD and GDM was due to the development of diabetes in the GDM group. In the current cohort, the 2-h plasma glucose value did not predict NAFLD in the multivariate analysis, which would have been expected if the association between NAFLD and GDM was only due to the development of diabetes. Furthermore, we are the first, to our knowledge, to carry out a 4-h OGTT in women with pGDM and NAFLD. An OGTT allows for the calculation of the degree of peripheral insulin resistance by the Matsuda index and determines the dynamic response of glucose, insulin, C-peptide, and glucagon by calculating the AUC during the 4-h glucose challenge. This provides a much more detailed image of the dynamics in glucose metabolism of these women than previously described and a chance to describe the impact of glucagon dynamics during an OGTT in the presence of NAFLD. The association between NAFLD and insulin resistance is well established in the general population, and the current findings support this association (8).

In a previous study from our group, fasting plasma glucagon levels were higher in patients with biopsy-proven NAFLD than in those with T2D and no evidence of NAFLD and in healthy control subjects (9). In the present cohort, fasting plasma glucagon was not associated with NAFLD, but the initial suppression of glucagon during the OGTT in the NAFLD pGDM group was significantly delayed. This phenomenon could reflect hepatic insulin resistance (9) or hepatic glucagon resistance (32), and these findings suggest an important role of NAFLD in the regulation of postabsorptive glucagon secretion.

During the screening visit, a thorough medical history was taken, including a record of recent and present use of hepatotoxic and lipogenic medication, and ALT, AST, and GGT levels were measured. Similarly to other studies (33), we found that women with NAFLD have higher plasma levels of ALT, AST, and GGT, although these were all within the normal range. These liver enzymes are markers of liver damage, and several studies have found ALT and GGT, even within the normal range, to predict diabetes (34). In the current study, none of the liver enzymes were significantly associated with the presence of NAFLD, which is partially in line with Forbes et al. (16), who found that ALT, but not AST and GGT, is associated with NAFLD. If our nonNAFLD pGDM group had been without mild stages of NAFLD, we might also have found an association between ALT and NAFLD, but this remains speculative. On the basis of the small and nonsignificant difference in AUDIT scores, we were able to rule out alcohol-induced liver damage.

The majority of women with pGDM in this study had abnormal glucose tolerance. Of note, we found no difference in the prevalence of prediabetes among the groups. This is in contrast to previous studies, which have demonstrated a higher prevalence of prediabetes in patients with NAFLD (5) and a strong correlation between T2D and NAFLD (3). Significantly higher fasting concentrations of C-peptide observed in the NAFLD pGDM group suggest that these patients' β-cells are still capable of adequately increasing insulin secretion at this time point, resulting in a nondiabetic glucose tolerance. This may explain why no difference in the prevalence of prediabetes was found between the pGDM groups and probably accounts for the similar disposition index. We found that the women with NAFLD had increased peripheral and hepatic insulin resistance. Hepatic insulin resistance upregulates lipogenic mechanisms in the

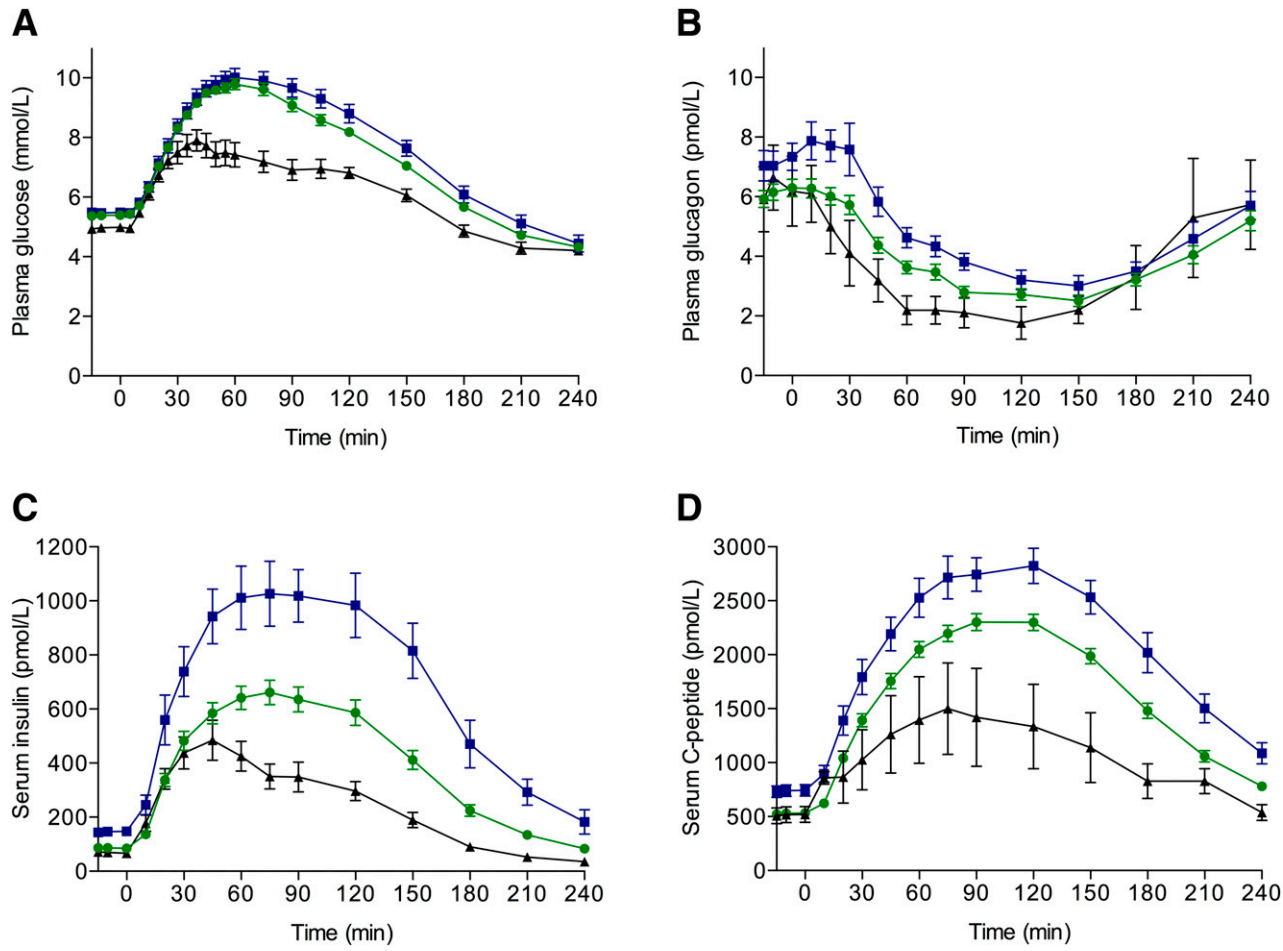

Figure 1—Responses of plasma glucose (*A*), plasma glucagon (*B*), serum insulin (*C*), and serum C-peptide (*D*) during OGTT. Black, control group; green, nonNAFLD pGDM group; blue, NAFLD pGDM group. Data are mean ± SEM.

liver, resulting in increased de novo lipogenesis, which further adds to fat accumulation in the liver and may start a vicious cycle (4–6). The current findings may reflect a higher risk of NAFLD in women with pGDM and predominantly insulin resistance than in women with pGDM and predominantly β-cell dysfunction.

The women were studied on average 5 years after their index pregnancy, and although NAFLD was associated with greater BMI, visceral fat mass, and waist circumference, only the latter parameter was independently associated with NAFLD. This may be explained by these parameters being closely interrelated. That said, previous studies have shown visceral adipose tissue to be more closely associated with the development of NAFLD than peripheral adipose tissue most likely because visceral adipose tissue is more metabolically active than subcutaneous adipose tissue (4).

It is well-known that NAFLD increases the risk of cardiovascular diseases, and the risk of cardiovascular-related death increases significantly with the presence of NAFLD in patients with T2D. This is believed to be mediated through a proatherogenic lipid profile: As the liver becomes insulin resistant and the influx of free fatty acids increases, the production of VLDL cholesterol increases, leading to a secondary lowering of HDL cholesterol and elevation of LDL cholesterol (4,35). Of note, although we excluded all women who were taking statins, we found that women with NAFLD had significantly lower HDL cholesterol, higher VLDL cholesterol, and higher triglyceride concentrations, which corroborates the results of Forbes et al. (16) but not Ajmera et al. (31). We found no differences in LDL cholesterol levels, which is similar to the finding of Ajmera et al. but contrasts that of Forbes et al. Blood pressure was lower in the control group, but no differences were found in the pGDM groups, and all groups were within normal ranges. No association was found between blood pressure and the presence of NAFLD, which is similar to Forbes et al. and Ajmera et al.

Early identification and diagnosis of high-risk individuals is needed to prevent the excess morbidity and mortality associated with NAFLD and T2D. The association between T2D and NAFLD and their shared etiology of insulin resistance is well-known, but the underlying pathophysiology is still unclear. Given their high risk of progression to T2D, women with pGDM provide a model for and an opportunity to study the early metabolic changes that precede both T2D and NAFLD (7,16,36). A recent study by De Souza et al. (37) showed how first-trimester ultrasound-defined NAFLD predicts dysglycemia in midpregnancy, and one could speculate that NAFLD may precede T2D in these women. We will prospectively follow this cohort for

Table 3—Logistic regression analysis

	Odds ratio	95% CI	*P* value
Weight (kg)	1.06	1.02–1.10	0.0013
BMI (kg/m^2)	1.24	1.11–1.41	0.0005
Waist circumference (cm)*	1.09	1.04–1.14	0.0003*
HDL cholesterol (mmol/L)	0.12	0.01–0.86	0.0474
VLDL cholesterol (mmol/L)	5.21	1.23–25.4	0.0287
Triglycerides (mmol/L)	2.28	1.20–4.66	0.0148
Visceral fat mass (kg)	6.69	2.69–19.9	0.0002
Android-to-gynoid fat ratio	6.05	2.12–23.3	0.0199
Total fat mass (%)	1.12	1.02–1.21	0.0215
ALT (units/L)	1.06	1.02–1.12	0.0107
AST (units/L)	1.05	1.01–1.10	0.0440
Matsuda index*	0.35	0.19–0.59	0.0003*
$HOMA2_{IR}$	4.24	3.06–36.8	0.0001
FLI	1.05	1.03–1.09	0.0002
Glucagon tAUC (0–45) (pmol/L × min)	1.01	1.00–1.01	0.0073

All significant univariable associations are listed. The following variables did not reach significance: glucose tolerance status, age, waist-to-hip ratio, HbA_{1c}, 2-h plasma glucose during OGTT, fasting plasma glucagon, family disposition to diabetes, use of hormonal contraceptive, total cholesterol, LDL cholesterol, AUDIT score, and presence of the metabolic syndrome.
*All significant univariable associations contributed in the multivariable analysis with backward elimination showing insulin resistance (odds ratio 0.44 [95% CI 0.23–0.75]; *P* = 0.0057) and waist circumference sensitivity (1.07 [1.02–1.12]; *P* = 0.0109) to be independently associated with the presence of NAFLD.

5 years to observe the incidence of both T2D and NAFLD as well as the progression of NAFLD over time and to detect parameters that predict both deteriorations. Treatment with thiazolidinediones has been shown to reduce not only steatosis but also hepatocellular damage in patients with NASH. The effect of thiazolidinediones may be mediated through reduced plasma lipid levels and altered fat topography combined with the insulin sensitizing effect, although concern exists about potential adverse effects, such as weight gain, fluid retention, heart failure, and bone fractures, when treating young and relatively healthy women (38,39). GLP-1 receptor agonists have also been shown to significantly lower liver enzymes, body weight, waist circumference, and visceral fat mass (40) as well as to reduce insulin resistance and lipotoxicity in patients with NASH (41). For this reason, one-half of the patients in the current study will be treated with a GLP-1 receptor agonist for 5 years (17).

In conclusion, we demonstrate that NAFLD is present in relatively young and not morbidly obese women with pGDM who are considered healthy apart from their increased risk for future diabetes. We also show that insulin resistance and waist circumference are independently and positively associated with the presence of ultrasound-defined NAFLD.

Acknowledgments. The authors thank the women who are participating in this study and are grateful to Nina Kjeldsen, Jytte Purtoft, Sisse M. Schmidt, and Inass A. Nachar (Center for Diabetes Research, Gentofte Hospital, University of Copenhagen) for laboratory assistance and to Lone Thielsen and Lene Albæk (Novo Nordisk Foundation Center for Basic Metabolic Research and Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen) and Novo Nordisk (Bagsværd, Denmark) for the supply of valine pyrrolidide.

Funding. The study was initiated by the authors and supported as an investigator-initiated study by unrestricted grants from Novo Nordisk and the Novo Nordisk Foundation and Danish Diabetes Academy. Research support was received from the AP Møller Foundation and Danielsen Foundation.

Duality of Interest. No potential conflicts of interests relevant to this article were reported.

Author Contributions. S.F. and L.V. contributed to the study design, performed the clinical experiments, researched data, and drafted, critically revised, and approved the final manuscript. C.A., E.S.A., and E.B. performed the clinical experiments, researched data, and critically revised and approved the final manuscript. C.S. performed the ultrasound and elastography and critically revised and approved the final manuscript. T.B. conducted the DXA scans and critically revised and approved the final manuscript. J.J.H. measured the incretin hormones and glucagon and critically revised and approved the final manuscript. J.A.S., T.D.C., E.R.M., and P.D. recruited the patients and critically revised and approved the final manuscript. L.L.G. and F.K.K. contributed to the study design, researched data, and critically revised and approved the final manuscript. T.V. designed the study, researched data, obtained funding, and critically revised and approved the final manuscript. T.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 74th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 13–17 June 2014; 75th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015; 50th European Association for the Study of Diabetes Annual Meeting, Vienna, Austria, 15–19 September 2014; and 51st European Association for the Study of Diabetes Annual Meeting, Stockholm, Sweden, 14–18 September 2015.

References

1. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–1231
2. Blachier M, Leleu H, Peck-Radosavljevic M, Valla D-C, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593–608
3. Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. Nat Rev Endocrinol 2011;7:456–465
4. Stefan N, Kantartzis K, Häring H-U. Causes and metabolic consequences of fatty liver. Endocr Rev 2008;29:939–960
5. Leite NC, Villela-Nogueira CA, Cardoso CRL, Salles GF. Non-alcoholic fatty liver disease and diabetes: from physiopathological interplay to diagnosis and treatment. World J Gastroenterol 2014;20:8377–8392
6. Stefan N, Häring H-U. The metabolically benign and malignant fatty liver. Diabetes 2011; 60:2011–2017
7. Pappachan JM, Antonio FA, Edavalath M, Mukherjee A. Non-alcoholic fatty liver disease: a diabetologist's perspective. Endocrine 2014; 45:344–353
8. Adams LA, Lymp JF, St. Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121
9. Junker AE, Gluud LL, van Hall G, Holst JJ, Knop FK, Vilsbøll T. Effects of glucagon-like peptide-1 on glucagon secretion in patients with non-alcoholic fatty liver disease. J Hepatol 2016;64: 908–915
10. Kobyliak N, Abenavoli L. The role of liver biopsy to assess non-alcoholic fatty liver disease. Rev Recent Clin Trials 2014;9:159–169
11. Buckley BS, Harreiter J, Damm P, et al.; DALI Core Investigator Group. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. A review. Diabet Med 2012;29:844–854
12. Lauenborg J, Hansen T, Jensen DM, et al. Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. Diabetes Care 2004;27:1194–1199
13. Kitzmiller JL, Dang-Kilduff L, Taslimi MM. Gestational diabetes after delivery. Short-term

management and long-term risks. Diabetes Care 2007;30(Suppl. 2):S225–S235
14. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 2002;25:1862–1868
15. Lauenborg J, Mathiesen E, Hansen T, et al. The prevalence of the metabolic syndrome in a Danish population of women with previous gestational diabetes mellitus is three-fold higher than in the general population. J Clin Endocrinol Metab 2005;90:4004–4010
16. Forbes S, Taylor-Robinson SD, Patel N, Allan P, Walker BR, Johnston DG. Increased prevalence of non-alcoholic fatty liver disease in European women with a history of gestational diabetes. Diabetologia 2011;54:641–647
17. Foghsgaard S, Vedtofte L, Mathiesen ER, et al. The effect of a glucagon-like peptide-1 receptor agonist on glucose tolerance in women with previous gestational diabetes mellitus: protocol for an investigator-initiated, randomised, placebo-controlled, double-blinded, parallel intervention trial. BMJ Open 2013;3:e003834
18. Sasso M, Miette V, Sandrin L, Beaugrand M. The controlled attenuation parameter (CAP): a novel tool for the non-invasive evaluation of steatosis using Fibroscan. Clin Res Hepatol Gastroenterol 2012;36:13–20
19. Kaul S, Rothney MP, Peters DM, et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring) 2012;20:1313–1318
20. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation [Internet], 2006. Geneva, Switzerland, World Health Organization. Available from http://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf. Accessed 1 March 2016
21. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–1470
22. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191–2192
23. Turner RC, Holman RR, Matthews D, Hockaday TD, Peto J. Insulin deficiency and insulin resistance interaction in diabetes: estimation of their relative contribution by feedback analysis from basal plasma insulin and glucose concentrations. Metabolism 1979;28:1086–1096
24. Alberti KGMM, Eckel RH, Grundy SM, et al.; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640–1645
25. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006;6:33
26. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption–II. Addiction 1993;88:791–804
27. Christensen M, Vedtofte L, Holst JJ, Vilsbøll T, Knop FK. Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. Diabetes 2011;60:3103–3109
28. Wewer Albrechtsen NJ, Bak MJ, Hartmann B, et al. Stability of glucagon-like peptide 1 and glucagon in human plasma. Endocr Connect 2015;4:50–57
29. Orskov C, Wettergren A, Holst JJ. Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. Diabetes 1993;42:658–661
30. Østoft SH, Bagger JI, Hansen T, et al. Incretin effect and glucagon responses to oral and intravenous glucose in patients with maturity-onset diabetes of the young—type 2 and type 3. Diabetes 2014;63:2838–2844
31. Ajmera VH, Gunderson EP, VanWagner LB, Lewis CE, Carr JJ, Terrault NA. Gestational diabetes mellitus is strongly associated with non-alcoholic fatty liver disease. Am J Gastroenterol 2016;111:658–664
32. Suppli MP, Lund A, Bagger JI, Vilsbøll T, Knop FK. Involvement of steatosis-induced glucagon resistance in hyperglucagonaemia. Med Hypotheses 2016;86:100–103
33. Bernsmeier C, Meyer-Gerspach AC, Blaser LS, et al. Glucose-induced glucagon-like peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. PLoS One 2014;9:e87488
34. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care 2009;32:741–750
35. Kotronen A, Peltonen M, Hakkarainen A, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137:865–872
36. Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest 2005;115:485–491
37. De Souza LR, Berger H, Retnakaran R, et al. Non-alcoholic fatty liver disease in early pregnancy predicts dysglycemia in mid-pregnancy: prospective study. Am J Gastroenterol 2016;111:665–670
38. Yki-Järvinen K. Thiazolidinediones and the liver in humans. Curr Opin Lipidol 2009;20:477–483
39. DeFronzo RA, Abdul-Ghani MA. Preservation of β-cell function: the key to diabetes prevention. J Clin Endocrinol Metab 2011;96:2354–2366
40. Carbone LJ, Angus PW, Yeomans ND. Incretin-based therapies for the treatment of non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Gastroenterol Hepatol 2016;31:23–31
41. Armstrong MJ, Hull D, Guo K, et al. Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis. J Hepatol 2016;64:399–408